

Amendment and Response

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Serial No.: 09/847,670

Confirmation No.: 4815

Filed: May 2, 2001

For: HEPATITIS C VIRUS HELICASE CRYSTALS, CRYSTALLOGRAPHIC STRUCTURE AND METHODS**Amendments to the Claims**

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1-30. (Canceled)

31. **(Withdrawn)** A method for crystallizing a Hepatitis C virus helicase molecule or molecular complex comprising growing a crystal from a precipitant solution comprising purified Hepatitis C virus helicase, about 3% by weight to about 14% by weight PEG, about 5% by weight to about 15% by weight DMSO, and about 0.05M to about 0.07M potassium phosphate.

32. **(Withdrawn)** A method for co-crystallizing a Hepatitis C virus helicase molecule and a ligand to yield a molecular complex, comprising:

exchanging purified Hepatitis C virus helicase into a solution comprising HEPES, EDTA, and dithiothreitol;

concentrating the Hepatitis C virus helicase to a concentration of about 12-16mg/mL;

combining concentrated Hepatitis C virus helicase with the ligand in a mixture comprising about 4% by weight to about 14% by weight PEG and about 5% by weight to about 15% by weight DMSO; and

growing a co-crystal by vapor diffusion.

33. **(Withdrawn)** The method of claim 32 wherein combining the concentrated Hepatitis C virus helicase with the ligand in a mixture comprising PEG and DMSO and growing the co-crystal are performed in the absence of potassium phosphate.

34. **(Withdrawn)** The method of claim 32 wherein the ligand binds to an NTP binding site on the Hepatitis C virus helicase.

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35. **(Withdrawn)** A method for crystallizing a Hepatitis C virus helicase molecule or molecular complex comprising growing a crystal by vapor diffusion with macro-seeding from a precipitant solution comprising purified Hepatitis C virus helicase, HEPES, and about 4% by weight to about 14% by weight mono-alkyl ether of PEG.
36. **(Withdrawn)** A method for co-crystallizing a Hepatitis C virus helicase molecule and a ligand to yield a molecular complex, comprising growing a crystal by vapor diffusion with macro-seeding from a precipitant solution comprising purified HCV helicase, HEPES, about 4% by weight to about 14% by weight mono-alkyl ether of PEG, and the ligand, wherein the ligand binds to at least one oligonucleotide binding site on the Hepatitis C virus helicase.
37. **(Withdrawn)** The method of claims 31-36 wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.
38. **(Original)** Crystalline Hepatitis C virus helicase comprising a tetragonal crystal having unit cell dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group $P4_1$; the unit cell containing two molecules in an asymmetric unit.
39. **(Currently Amended)** The crystalline Hepatitis C virus helicase of claim 38 wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.
40. **(Original)** Crystalline Hepatitis C virus helicase comprising an orthorhombic crystal characterized by unit cell dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group $P2_12_12_1$; the unit cell containing one molecule in the asymmetric unit.
41. **(Currently Amended)** The crystalline Hepatitis C virus helicase of claim 40 wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.

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42. **(Previously Presented)** Crystalline Hepatitis C virus helicase having amino acid sequence SEQ ID NO:1.

43. **(Original)** A composition comprising crystalline Hepatitis C virus helicase of any of claims 38-42.

44-46. **(Canceled)**

47. **(Withdrawn)** A method for incorporating a chemical entity in a crystal comprising placing a tetragonal crystal of Hepatitis C virus helicase having unit cell dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group P4₁ in an aqueous solution comprising about 1mM to about 10mM chemical entity, and 0% by weight to about 15% by weight DMSO.

48. **(Withdrawn)** A method for incorporating a chemical entity in a crystal comprising placing an orthorhombic crystal of Hepatitis C virus helicase having unit cell dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group P2₁2₁2 in an aqueous solution comprising about 1mM to about 10mM chemical entity, and 0% by weight to about 15% by weight DMSO.

49. **(Currently Amended)** Crystalline Hepatitis C virus helicase wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.

50. **(New)** A crystal of Hepatitis C virus helicase, wherein the Hepatitis C virus helicase comprises amino acid sequence SEQ ID NO:1.

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51. (New) A crystal of Hepatitis C virus helicase, wherein the Hepatitis C virus helicase comprises amino acid sequence SEQ ID NO:1, with the proviso that at least one cysteine or methionine is replaced with selenocysteine or selenomethionine, respectively.
52. (New) A crystal of Hepatitis C virus helicase, wherein the Hepatitis C virus helicase consists of amino acid sequence SEQ ID NO:1.
53. (New) A crystal of Hepatitis C virus helicase comprising a unit cell having dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group $P4_1$; the unit cell containing two molecules in an asymmetric unit.
54. (New) A crystal of Hepatitis C virus helicase comprising a unit cell having dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group $P2_12_12_1$; the unit cell containing one molecule in the asymmetric unit.
55. (New) A crystal of Hepatitis C virus helicase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 1.
56. (New) A crystal of Hepatitis C virus helicase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 2.
57. (New) A crystal of Hepatitis C virus helicase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 3.
58. (New) A crystal of Hepatitis C virus helicase prepared by a method comprising growing a crystal from a precipitant solution comprising purified Hepatitis C virus helicase, about 3% by weight to about 14% by weight PEG, about 5% by weight to about 15% by weight DMSO, and about 0.05M to about 0.07M potassium phosphate.

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59. (New) A crystal of Hepatitis C virus helicase prepared by a method comprising growing a crystal by vapor diffusion with macro-seeding from a precipitant solution comprising purified Hepatitis C virus helicase, HEPES, and about 4% by weight to about 14% by weight mono-alkyl ether of PEG.